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Does the azimuth orientation of Norway spruce (*Picea abies*/L./Karst.) branches within sunlit crown part influence the heterogeneity of biochemical, structural and spectral characteristics of needles?

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Abstract

The goal of this study was to determine if selected biochemical, structural and spectral properties of Norway spruce needles are influenced by the azimuth orientation of the branch. Three youngest needle age classes from 20 mature (100 years old or older) Norway spruce trees were sampled from upper branches of the sunlit production crown part from each of the 4 cardinal azimuth orientations. Photosynthetic pigments, soluble phenolic compounds and selected spectral and structural characteristics were determined for each needle age class. The content of photosynthetic pigments and soluble phenolic compounds did not differ among needles from different azimuth-oriented branches, nor did the optical reflectance indices Normalized Difference Vegetation Index (NDVI), Transformed Chlorophyll Absorption in Reflectance Index (TCARI)/Optimized Soil-Adjusted Vegetation Index (OSAVI), Red Edge Inflection Point (REIP) and Landsat Thematic Mapper bands 5 and 4 (TM5/TM4). No variation in volume properties, tissue volume proportions and cross-section shape characteristics of 3rd-year needles rejected our hypothesis that there would be variation in needle structural properties according to the azimuth orientation of branches. Consequently, we concluded that a random sampling of similar-aged needles within the sunlit production crown part may be used to study biochemical or structural and spectral needle properties of a mature Norway spruce growing in forest stands without a significant slope. In addition, the results obtained from a branch of one azimuth orientation should be representative for the whole sunlit portion of the crown. Consequences of these findings for Norway spruce health monitoring using remote sensing techniques are discussed.

Keywords: Branch azimuth orientation; Norway spruce; Chlorophyll; Phenolic compounds; Needle structure; Spectral reflectance indices; Remote sensing

1. Introduction

The spatial heterogeneity of environmental factors acting within a tree canopy increases with tree height and age. Different microclimates within the crown of conifers, sometimes synergistically acting with air pollution, usually cause modifications in shoot architecture and foliar properties such as leaf anatomy, photosynthetic activity or biomass allocation. Significant scientific attention has focused on the effect of different irradiation in the vertical crown gradient connected with foliar shade adaptations. Steep vertical gradients of photosynthetically active radiation were measured in Norway spruce crowns (Špunda et al., 1998), and young Norway spruce canopies under direct sunshine exhibit shaded needles in the lower parts of crown that are exposed to approximately 10% photosynthetic photon flux density as compared to exposed needles in upper canopy layers (Kalina et al., 2001). According to structural parameters of needles and shoots within the Norway spruce canopy profile can be differentiated between sun- and shade-adapted zones (Pokorný et al., 2004).

Different intensity of irradiation causes modification in needle anatomy such as increased thickness and width of Norway

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spruce sun needles (Niinemets, 1997) and the increased thickness of mesophyll in cross-section displayed by sun needles of *Abies grandis* and *Picea engelmanii* (Youngblood and Ferguson, 2003). The physiological processes of photosynthesis are also remarkably influenced by intensity of irradiation (Špunda et al., 1998). Not only do needle characteristics change with shade adaptations, but shoot geometry and morphology in the conifer canopy is dependent on the irradiance gradient. Greater clumping of needles (number of needles per unit shoot length) was observed by Niinemets and Kull (1995) with increasing irradiance and height of Norway spruce crowns. Sunlit shoots of Norway spruce typically have higher needle area per unit shoot length than shaded shoots, and the orientation of needles around the shoot axis is more uniform in contrast to shaded shoots where needles are arranged in a laminar fashion (Stenberg et al., 1999). In addition to needle differentiation caused by vertical irradiance gradient in the tree crown, in coniferous species with greater needle retention, increasing age of needles is another important factor which influences foliar properties (e.g. Rock et al., 1994; Soukupová et al., 2001).

Some of the above mentioned foliar properties are used as non-specific markers of initial tree damage (Wild and Schmitt, 1995; Soukupová et al., 2001), and thus the sampling design and the implied question of representative nature of the data obtained from samples is important. To use the biochemical, structural and spectral needle properties as diagnostic tools for tree physiological status assessment properly, it is necessary to know how these foliar parameters vary within an individual tree crown. The spectral properties of leaves, used as a non-destructive method for monitoring forest health (e.g. Rock et al., 1986, 1988; Albrechtová et al., 2001), are dependent on biochemical and structural foliar parameters (Gates, 1970). Therefore, biochemical, structural and spectral foliar parameters are often used as ground truth for remote sensing vegetation monitoring analyses (e.g. Rock et al., 1988; Albrechtová et al., 2001; Entcheva Campbell et al., 2004). For proper interpretation and verification of remote sensing data and results regarding the physiological status of foliage, it is crucial to know if there is any kind of heterogeneity in such ground truth within the sunlit portion of a single crown.

On the basis of branching type zonation (Gruber, 1994), three parts of the crown of a mature Norway spruce tree can be distinguished according to function (Cudlín et al., 2001). The upper-most portion of the crown, representing about 5–10% of the crown height, is known as juvenile and consists of sunlit branches. The growth activity of this juvenile crown part is very intensive and its main function consists of new needle and branch production. The middle portion of the crown is known as the production part, because the majority of assimilates produced by the spruce crown are produced here. The lower part of the crown is heavily shaded and is called the saturation part and this part contributing only slightly to assimilate production. Because of the juvenile crown branch architecture, shading is very limited in the upper production crown, where the pyramidal shape of a Norway spruce crown merges into the cylindrical shape. Considering these facts, we chose to study the upper production crown as representative for the sunlit part of a tree crown.

While information about the vertical heterogeneity within the tree crown related to different solar irradiance is available in the literature, less information is available concerning the potential horizontal heterogeneity of foliar properties connected to azimuth orientation of branches. Only limited studies of horizontal distribution of branch and needle parameters have been conducted, although sampling designs often reflect assumed heterogeneity. For example, differences dependent on branch azimuth orientation were described for catalase activity in Norway spruce needles, where the enzyme activity was higher in needles from north-facing branches than in south-facing branches (Schittenhelm et al., 1994). The activity of catalase in Norway spruce needles decreased under stress conditions or in response to increased light intensity, and thus was classified as photoinactivation (Schittenhelm et al., 1994). Information about assumed heterogeneity related to branch azimuth orientation is important for planning experimental designs of stress physiological studies. When using remote sensing methods for forest health monitoring, the question about heterogeneity in sunlit portion of the tree crown becomes even more important since needles sampled are analyzed as a source of ground truth or calibration information for interpreting remote sensing techniques. In addition, if azimuthal heterogeneity is documented for sunlit portions of Norway spruce canopies, such variation may need to be incorporated into the interpretation of hyperspatial/hyperspectral remote sensing data sets.

We tested the hypothesis that the sun-exposed needles from the upper production crown of Norway spruce will display a variability dependent on the azimuth orientation of the branch. The aim of this study was to investigate whether selected biochemical, structural and selected spectral properties of Norway spruce needles are influenced by the azimuth orientation of the branch within the sunlit part of the upper production crown part.

2. Material studied and methods

2.1. Site description and sampling setup

The study site was located in the Šumava Mts. in southern Bohemia (Czech Republic), in the National Park Šumava, located near Modrava (48°59′N, 13°28′E), at an altitude of 1075 m a.s.l., on a slope of almost zero. All trees included in the study grew in a closed canopy of homogenous, even-aged (over 100 years old) Norway spruce.

Twenty dominant and co-dominant trees with total defoliation 40% or less and without irreversible damage were selected for study. Sunlit branches growing in the four cardinal azimuth directions were sampled from the upper production part of each canopy by a tree climber equipped with a handsaw. Needles of the youngest three age classes (1st, 2nd and 3rd year) were sampled. Needles for foliar biochemical and structural analyses were cut off immediately in the field and transported in a portable cooler to the laboratory where they were stored at −70 °C until processed. Shoots collected for spectral analysis were immediately sealed in plastic bags with wet paper towels, placed in coolers containing frozen "blue ice" packets, and taken to laboratory for spectral scanning. Three successive sample sets each

Table 1
Dates of sample collections, number of trees sampled and tree characteristics

Sampling date	Number of trees	Total defoliation (%)	Analyses
September 2001	8	<45	Pigments, phenolics, structural parameters
November 2001	5	<45	Pigments, phenolics, spectral
September 2002	7	<35	Pigments

time from different trees were acquired: mid-September 2001, early November 2001 and mid-September 2002 (Table 1).

2.2. Foliar chemistry

Chlorophyll *a*, chlorophyll *b* and total carotenoids were extracted in dimethylformamide (DMF) according to the method of Porra et al. (1989). The amount of photosynthetic pigments was determined spectrophotometrically according to equations of Welburn (1994). For further details, see Soukupová et al. (2001). The amount of pigments was expressed as weight of pigment per gram of needle dry weight.

Soluble phenolic compounds were extracted in boiling methanol, detected using the Folin-Ciocalteau reagent and determined spectrophotometrically using gallic acid as a standard (Singleton and Rossi, 1965). The amount of phenolic compounds was expressed as weight of soluble phenolics per gram of needle fresh weight.

2.3. Needle structural analyses

Third-year needles were collected in the field as described above and kept frozen until processed. Transverse needle sections were prepared according to the principle of systematic uniform random sampling (Kubínová, 1991; Fig. 1). An average of five hand microtome sections about 50 μm thick was made along the longitudinal needle axis at an interval *T*. To achieve at least five representative cross-sections along the needle, the distance *T* between two subsequent sections was established to be 1/5 of mean needle length that corresponds to 2 mm. Random position *z* of the first section was selected at the following distances from the needle apex: 0, 0.5, 1 and 1.5 mm. Digital images of sections were acquired using an Olympus Camedia 5050 camera at a magnification of 400× using a light transmission microscope (Olympus BX 50). The area of the whole sections and needle tissues was determined using the image analysis software Lucia G (LIM, Prague). The total needle volume and volume of mesophyll tissue, volume of dermal tissues (epidermis and hypodermis together), and of central cylinder were determined by the Cavalieri method in combination with the point-counting method (Kubínová, 1993). The volume proportion of individual tissues was expressed as percent of the total needle volume.

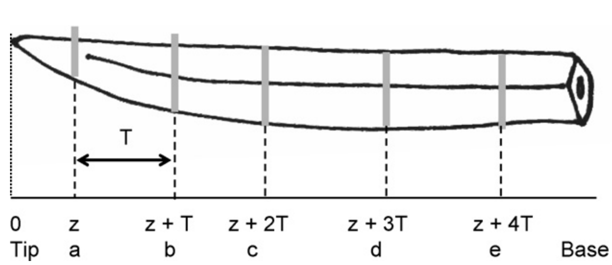

Fig. 1. Systematic uniform random sampling of needle sections ($T = 2$ mm) is the distance between transverse sections (mm); (z) is the random position from the set {0.5, 1, 1.5, 2 mm} determining the distance of the first section from needle apex. Transverse sections were made in the positions z, $z + T$, $z + 2T$, ... and labeled (a–e) from tip to base.

2.4. Spectral analyses

Shoots of 1st-, 2nd- and 3rd-year needle age classes with needles attached were separated and placed in a black weighing dish as an optically dense multiple layer of several shoots (see Rock et al., 1994 for more detail). The directional reflectance of the shoot layer at spectral interval of 400–2500 nm was measured by a spectroradiometer VIRIS (GER 2600, USA). The hemispherical tungsten light source provided artificial illumination following the method of Rock et al. (1994). The sensor head and light source were each situated 50 cm from the sample, at a standard angle of illumination direction of 45° and viewing direction of the sensor was set in nadir position (perpendicular to the sample) simulating setup of most airborne acquisitions. The entire field of view of the sensor was covered by the shoot sample. A standard spectralon panel (Spectrolon-Labsphere Inc., North Sutton, NH, USA) with nearly 100% reflectance at all wavelengths was used as a white Lambertian reference. Multiple sample and reference scans were made and divided to produce a reflectance measurement for each sample. Reflectance of each shoot sample was measured three times with rotation of 90° of the sample dish after each measurement, and all three measurements were averaged into a single reflectance curve representative of each needle age class.

The following optical indices were selected for spectral analyses of the spruce shoot reflectance curves: the ratio TCARI/OSAVI (Haboudane et al., 2002), the Red Edge Inflection Point (REIP) (Rock et al., 1988), Normalized Difference Vegetation Index (NDVI) (Vogelmann et al., 1993) and ratio of simulated Landsat Thematic Mapper bands 5 and 4 (TM5/TM4) (Rock et al., 1986).

Haboudane et al. (2002) proposed the ratio of Transformed Chlorophyll Absorption in Reflectance Index (TCARI) and Optimized Soil-Adjusted Vegetation Index (OSAVI) (Rondeaux et al., 1996) as strongly sensitive to chlorophyll concentration and in addition very resistant to the variations of Leaf Area Index (LAI) and solar zenith angle at the canopy level. The TCARI was developed as a transformed variant of the chlorophyll index Modified Chlorophyll Absorption in Reflectance Index (MCARI) (Daughtry et al., 2000), and was defined as follows:

$$\mathrm{TCARI} = 3\left[(\rho_{700} - \rho_{670}) - 0.2(\rho_{700} - \rho_{550})\left(\frac{\rho_{700}}{\rho_{670}}\right)\right] \quad (1)$$

where ρ_j is the reflectance value at the jth wavelength in nanometres. The OSAVI index belongs to the family of soil line vegetation indices (Steven, 1998), which can be computed by the equation:

$$\mathrm{OSAVI} = \frac{(1 + 0.16)(\rho_{800} - \rho_{670})}{\rho_{800} + \rho_{670} + 0.16}. \quad (2)$$

The REIP is used to quantify the position of the inflection point of the sharp change in the vegetation reflectance curve between 680 and 750 nm. A shift of the REIP position towards shorter wavelengths, due to in chlorophyll concentration and changes in inner leaf structure, indicates early vegetation stress (Rock et al., 1988; Entcheva Campbell et al., 2004). Traditionally, the wavelength position of the REIP can be determined as a maximum value of the first derivative reflectance curve (Horler et al., 1983; Lamb et al., 2002). The first-difference transformation of measured reflectance between 680 and 750 nm was computed from:

$$D_i = \frac{\rho_{j+1} - \rho_j}{\lambda_{j+1} - \lambda_j} \quad (3)$$

where D_i is the first derivative at a wavelength λ_i (i is a midpoint between bands j and $j+1$), ρ_j and ρ_{j+1} the reflectance values at the j and $j+1$ bands, and λ_j and λ_{j+1} are the wavelengths of the j and $j+1$ bands. Consequently, a maximum value of first derivative D_{REP} was located, and the corresponding wavelength λ_{REP} was assigned as the REIP position.

The Normalized Difference Vegetation Index was originally designed to distinguish vegetation from other objects (bare soil, snow, water and clouds) (Lillesand and Kiefer, 1994; Williams, 1995) based on reflectance differences at visible and NIR wavelengths caused by different chemical and structural characteristics of surfaces. In our study, it provides information about the amount of green biomass (green foliage) against the brown biomass (woody parts) within the field of view of the spectrometer used to measure shoot samples. NDVI is the normalized ratio of the amounts of reflectance in the near infrared (ρ_{NIR}) and red (ρ_{RED}) portions of the electromagnetic spectrum, calculated using the formula:

$$\mathrm{NDVI} = \frac{\rho_{\mathrm{NIR}} - \rho_{\mathrm{RED}}}{\rho_{\mathrm{NIR}} + \rho_{\mathrm{RED}}}. \quad (4)$$

Finally, two broader spectral bands, simulating fourth and fifth bands of the Landsat Thematic Mapper (TM) scanner, were generated from the measured reflectance signatures between the wavelengths of 0.76–0.90 μm (TM band 4) and 1.55–1.75 μm (TM band 5). A simple ratio TM5/TM4 (Rock et al., 1986; Ardö et al., 1997) reveals basic information about the leaf water content of the samples investigated.

2.5. Statistical analyses

Statistical comparisons were made using NCSS 6.0 software (NCSS, Kaysville, UT, USA). Normally distributed data were analyzed by the one-way ANOVA analysis and differences among the azimuth directions of the branches were detected using the Tukey–Kramer test. Otherwise distributed data were analyzed using the Kruskal–Wallis test and Kruskal–Wallis Z-tests. The linear regression was used to determine relationships between foliar chemistry and spectral indices.

3. Results

3.1. Foliar chemistry

Photosynthetic pigments – the average amount of chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoids – in the first three needle age classes did not show significant statistical difference for needles sampled from different azimuth-oriented branches (Fig. 2). The amount of photosynthetic pigments was age dependent and increased with needle age (Table 2). Identical results were observed for all three data sets obtained from sampling on September 2001, November 2001 and September 2002.

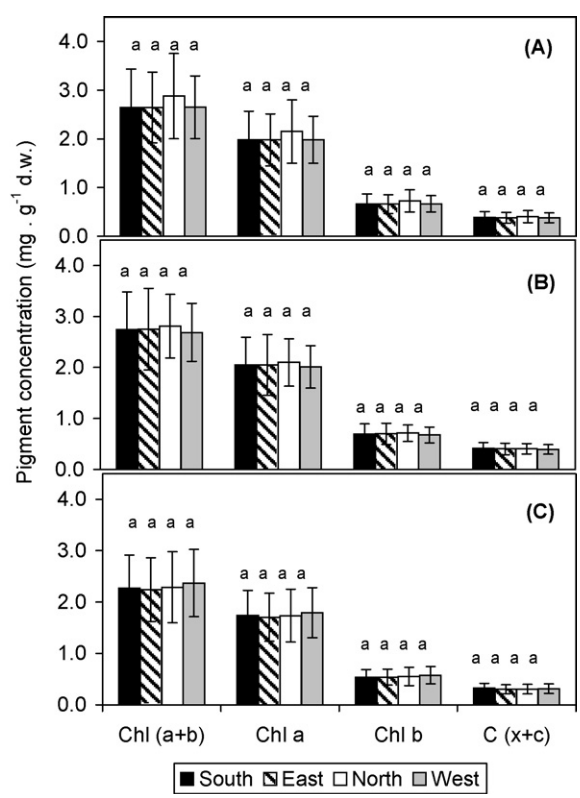

Fig. 2. Concentrations of photosynthetic pigments in needles sampled from different azimuth orientations. Means for the youngest three needle age classes. Expressed as mg of pigment per 1 g needle dry weight. Collection dates: (A) September 2001, (B) November 2001, (C) September 2002. Chl ($a+b$): total chlorophylls; Chl a: chlorophyll a; Chl b: chlorophyll b; C ($x+c$): total carotenoids. Bars represent standard deviations. Equal letters above the columns show no significant difference at $\alpha = 0.05$, based on one-way ANOVA.

Table 2
Age dependence of needle biochemical and shoot spectral reflectance properties

	Needle/shoot age class			Branch orientation
	1st	2nd	3rd	
September 2001				
Chlorophyll *a* (mg g^{-1} d.w.)	1.53 (±0.29) b	2.19 (±0.52) a	2.35 (±0.47) a	ns
Chlorophyll *b* (mg g^{-1} d.w.)	0.53 (±0.12) b	0.71 (±0.19) a	0.79 (±0.18) a	ns
Total chlorophyll (mg g^{-1} d.w.)	2.05 (±0.40) b	2.91 (±0.71) a	3.15 (±0.64) a	ns
Total carotenoids (mg g^{-1} d.w.)	0.27 (±0.04) c	0.42 (±0.09) b	0.47 (±0.08) a	ns
Soluble phenolic compounds (mg g^{-1} d.w.)	102.25 (±25.45) b	151.52 (±32.50) a	169.10 (±30.22) a	ns
November 2001				
Chlorophyll *a* (mg g^{-1} d.w.)	1.68 (±0.39) b	2.16 (±0.42) a	2.32 (±0.44) a	ns
Chlorophyll *b* (mg g^{-1} d.w.)	0.58 (±0.16) b	0.71 (±0.15) a	0.78 (±0.16) a	ns
Total chlorophyll (mg g^{-1} d.w.)	2.26 (±0.55) b	2.88 (±0.57) a	3.10 (±0.60) a	ns
Total carotenoids (mg g^{-1} d.w.)	0.31 (±0.07) b	0.43 (±0.08) a	0.48 (±0.08) a	ns
Soluble phenolic compounds (mg g^{-1} d.w.)	70.04 (±7.97) c	81.16 (±9.02) b	87.45 (±6.49) a	ns
Water content %	59.33 (±1.27) a	55.73 (±0.75) b	55.2 (±0.86) b	ns
NDVI	0.84 (±0.02) a	0.83 (±0.02) a	0.84 (±0.02) a	ns
TCARI/OSAVI	11.06 (±1.60) a	8.63 (±1.23) b	7.96 (±1.15) b	ns
REIP (nm)	722.71 (±1.86) a	732.14 (±2.42) a	723.84 (±2.17) a	ns
TM5/TM4	0.39 (±0.04) b	0.48 (±0.04) a	0.49 (±0.02) a	ns
September 2002				
Chlorophyll *a* (mg g^{-1} d.w.)	1.33 (±0.27) c	1.73 (±0.35) b	2.17 (±0.39) a	ns
Chlorophyll *b* (mg g^{-1} d.w.)	0.42 (±0.09) c	0.54 (±0.12) b	0.69 (±0.14) a	ns
Total chlorophyll (mg g^{-1} d.w.)	1.75 (±0.36) c	2.27 (±0.47) b	2.86 (±0.53) a	ns
Total carotenoids (mg g^{-1} d.w.)	0.23 (±0.04) c	0.31 (±0.06) b	0.40 (±0.07) a	ns

Note: All values listed are means (±S.D.) of four examined azimuth-oriented branches. September 2001, $n = 7$ trees; November 2001, $n = 5$ trees; September 2002, $n = 8$ trees. Values for characteristics with identical letters show no significant differences. "ns" in Branch orientation column refers to no significant differences among different azimuth-oriented branches. One-way ANOVA, $\alpha = 0.05$. Tukey–Kramer or Kruskal–Wallis *Z*-tests were used for multiple comparisons.

Regarding amounts of soluble phenolic compounds, the statistical tests showed that the average amount of soluble phenolic compounds of the three needle age classes was not significantly different between the needles from different azimuth-oriented branches (Fig. 3). Regardless the branch orientation, the content of soluble phenolic compounds in needles increased with needle age (Table 2). Identical observations were obtained for both set of samples from September and November 2001.

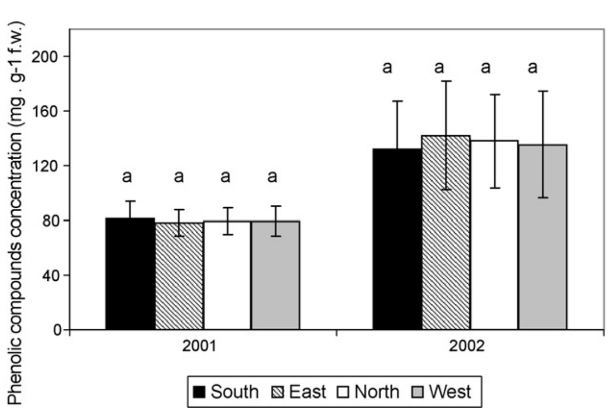

Fig. 3. Concentration of soluble phenolic compounds in needles sampled from different azimuth orientations. Means for the youngest three needle age classes. Expressed as mg of phenolics per 1 g needle fresh weight. Bars represent standard deviations. Equal letters above the columns show no significant difference at $\alpha = 0.05$, based on one-way ANOVA.

3.2. Needle structural analyses

Needles of different azimuth-oriented branches did not differ significantly in either volume characteristics—total needle volume, volume of mesophyll tissue, dermal tissues and central cylinder or in the relative volume proportions of the above tissues (Table 3). The shape characteristics of needle cross-section – perimeter and circularity – were not significantly different for needles of all azimuth orientations (Table 3).

The structural parameters of needle cross-sections were related to the position along the longitudinal needle axis (Fig. 4).

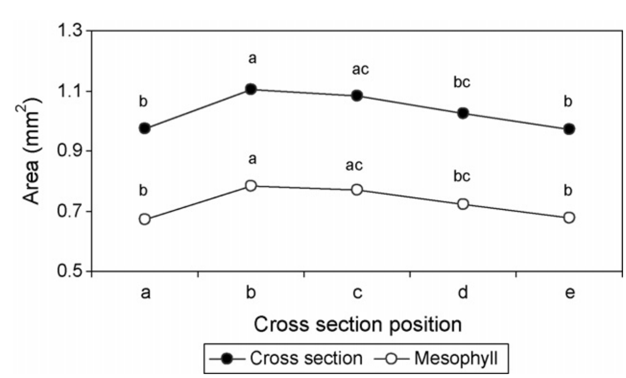

Fig. 4. Gradient of selected structural parameters along the longitudinal needle axis. Closed circles refer to cross-section area, open circles to mesophyll area on the cross-section. Position of cross-section: (a) tip and (e) base of needle; $n = 7$. Different letters above the columns show significant difference at $\alpha = 0.05$, based on one-way ANOVA. For multiple comparisons Tukey–Kramer test was used.

Table 3
Structural properties of needles collected from different azimuth-oriented branches of Norway spruce

Needle structural properties	Branch azimuth orientation			
	South	East	North	West
Needle volume (mm^3)	10.32 (±2.68) a	10.17 (±2.62) a	9.00 (±1.87) a	9.70 (±1.33) a
Mesophyll volume (mm^3)	7.25 (±1.91) a	7.18 (±1.91) a	6.32 (±1.39) a	6.78 (±0.98) a
Dermal tissues volume (mm^3)	1.85 (±0.48) a	1.84 (±0.40) a	1.66 (±0.31) a	1.77 (±0.19) a
Central cylinder volume (mm^3)	0.93 (±0.29) a	0.87 (±0.30) a	0.76 (±0.17) a	0.88 (±0.23) a
Mesophyll (% of total needle volume)	70.35 (±1.31) a	70.48 (±1.53) a	70.10 (±1.44) a	69.93 (±2.03) a
Dermal tissues (% of total needle volume)	17.97 (±0.53) a	18.27 (±1.46) a	18.59 (±1.09) a	18.32 (±0.83) a
Central cylinder (% of total needle volume)	8.97 (±1.16) a	8.49 (±1.06) a	8.43 (±0.81) a	9.01 (±1.74) a
Cross-section circularity	0.82 (±0.05) a	0.80 (±0.04) a	0.83 (±0.03) a	0.81 (±0.04) a
Cross-section perimeter (mm)	3.97 (±0.57) a	3.93 (±0.50) a	3.63 (±0.20) a	3.86 (±0.30) a

Note: All measurements were done for 3 years old needles collected in September 2001. Values listed are means (±S.D.). $n = 7$ trees. Values for characteristics with identical letters show no significant differences among different azimuth-oriented branches. One-way ANOVA, $\alpha = 0.05$.

The area of the cross-section and the area of mesophyll were significantly lower in positions near to tip and base of needle in comparison to the middle positions. Area proportions of mesophyll in cross-section and cross-section perimeters displayed the same pattern as the parameters mentioned above. In contrast to the above measurements, the area of dermal tissues and the relative proportion of dermal tissues in cross-section were higher in positions near to tip and base of needle in comparison to the middle positions. Needles collected from all trees and all azimuth orientations exhibited the identical pattern of structural parameters along the needle longitudinal axis.

3.3. Laboratory spectral indices—NDVI, TCARI/OSAVI, REIP and TM 5/4

The average values of the spectral indexes NDVI, TCARI/OSAVI, REIP and TM 5/4 did not show significant differences among shoots sampled from different azimuth-oriented branches, even when separate needle age classes were considered (Table 4). The NDVI did not display age dependence (Table 2), which indicates that comparable ratios of green and non-green biomass were present in samples of all three needle age classes. Values of the TCARI/OSAVI index correlated significantly with total chlorophyll content ($R^2 = 0.64$; Fig. 5). A significant correlation was also observed in the case of the REIP and total chlorophyll, but the correlation coefficient was lower ($R^2 = 0.26$; Fig. 5). The TM5/TM4 values for current year shoots (1st-year needles) were significantly lower than values for two older needle age classes, indicating higher water content when compared to older needles (Table 2). This phenomenon was also observed for shoots from all branch orientation. Significant correlations were observed between the amount of water in needles (percent of fresh mass) and TM5/TM4 ($R = -0.71$; Fig. 6).

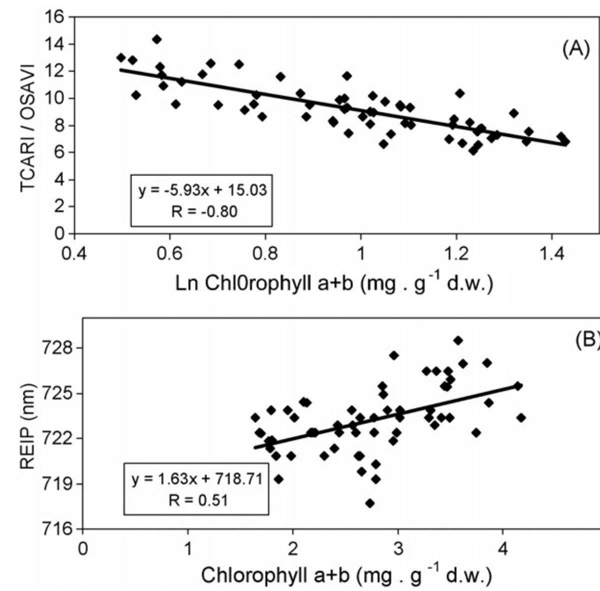

Fig. 5. Relationship between total chlorophyll concentration and spectral indices. (A) Total chlorophyll concentration and TCARI/OSAVI ratio in the youngest three needle age classes. Linear regression, $p < 0.05$, $R = -0.80$. (B) Total chlorophyll concentration and REIP in three youngest needle age classes. Linear regression, $p < 0.05$, $R = 0.51$. Collection date, November 2001. $n = 90$.

Table 4
Spectral indices derived from reflectance of shoots collected from different azimuth-oriented branches of Norway spruce

Spectral indices	Branch azimuth orientation			
	South	East	North	West
NDVI	0.84 (±0.02) a	0.84 (±0.03) a	0.83 (±0.02) a	0.83 (±0.01) a
TCARI/OSAVI	8.98 (±1.68) a	9.31 (±1.86) a	9.15 (±1.74) a	8.80 (±1.70) a
REIP (nm)	723.19 (±1.65) a	723.53 (±2.17) a	722.89 (±2.95) a	723.16 (±1.88) a
TM5/TM4	0.44 (±0.06) a	0.43 (±0.06) a	0.47 (±0.06) a	0.47 (±0.05) a

Note: All measurements were done for shoots collected in November 2001. Values listed are means for three shoot classes (±S.D.). $n = 5$ trees. Values for characteristics with identical letters show no significant differences. One-way ANOVA, $\alpha = 0.05$.

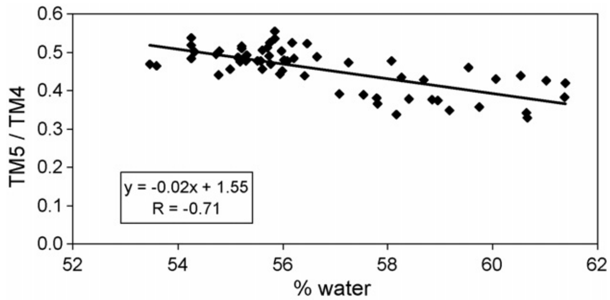

Fig. 6. Relationship between relative water content and TM5/TM4 in the youngest three needle age classes. Linear regression, $p < 0.05$, $R = -0.71$. Collection date, November 2001. $n = 90$.

4. Discussion

We observed no differences in all of the parameters studied for needles from different azimuth-oriented branches and this does not support our hypothesis that heterogeneity of needles would exist due to azimuth orientation of branches, if they are sampled the on the same sunlit canopy level.

The majority of studies involving sampling the trees under field conditions do not provide any information about azimuth orientation of sampled shoots or needles. Another approach is to take samples from branches uniformly oriented, usually from south (e.g. Tegischer et al., 2002; Vrchotová et al., 2004) in order to minimize possible microclimatic differences within the stand or canopy, or mixed sampling is accomplished from different azimuth-oriented branches (Shumejko et al., 1996).

Accumulation of soluble phenolic compounds in Norway spruce needles is considered as part of a non-specific stress reaction (Wild and Schmitt, 1995). Moreover, phenolic compounds accumulate in needles during high UV-B irradiance conditions since they are involved in UV-B protection (Laakso et al., 2000). Indistinguishable differences in amounts of phenolic compounds detected in needles of different azimuth-oriented branches suggest identical levels of stress in all of four azimuth directions. According to Vrchotová et al. (2004) some of methanol extractable phenolic compounds of Norway spruce needles such as catechin, may also display variability due to the different availability of solar radiation. Shaded needles contained lover values of catechin than sunlit needles. On the contrary, the amount of another methanol extractable phenolic compound, 4-hydroxyacetophenone, was not influenced by exposure to radiation (Vrchotová et al., 2004). In our study, we assessed all methanol extractable phenolic compounds together without specific identification of individual components. Therefore, the potential variation in content of individual components may be masked by other prevailing components without variation within azimuth orientations. This can be avoided by precise separation of individual phenolic components using HPLC.

It is a commonly known fact that amount of photosynthetic pigments increases with the needle age of Norway spruce (Rock et al., 1988, 1994; Köstner et al., 1990; Soukupová et al., 2000) and other coniferous species, too. A similar trend was observed for the content of soluble phenolics (Giertych et al., 1999; Soukupová et al., 2001). The comparable results for photosynthetic pigments and soluble phenolics support the interpretation of a general horizontal homogeneity of these metabolites in even-aged sunlit Norway spruce needles.

Both structural and biochemical properties of Norway spruce needles may be influenced by the intensity and availability of solar irradiation. Larger needle width and thickness have been observed for sun needles of Norway spruce in comparison to shade needles (Niinemets, 1997). However, the identical tissue volumes, volume proportions, circularity and cross-section perimeter of our needle samples facing four different azimuth orientations do not support the occurrence of any structural differentiation associated with varying orientation of even-aged needles from shoots of identical ontogenetic origin. Thus, it can be implied that different azimuth orientation of branches does not induce different microclimatic and irradiance conditions which would affect needle biochemical and structural features leading to differentiation into sun and shade foliage.

A common practice of evaluating structural characteristics of needles uses only sections taken from the middle part of needles (e.g. Leal and Thomas, 2003; Apple et al., 2002). This approach is acceptable if we do not derive mean parameters for the entire needle based on observations at the middle needle segment only. The principle of systematic uniform random sampling of sections along the needle axis allows the inclusion of a potential gradient in anatomical characteristics within the needle. Additionally, in combination with the Cavalieri method, it allows an unbiased volume estimation of needle structural components (Kubínová, 1993), which cannot be derived with biased sampling.

Knowledge of the horizontal variability of reflectance data within the crown is a crucial point for studies using high spatial resolution (hyperspatial) and high spectral resolution (hyperspectral) remote sensing data for the purpose of forest monitoring (e.g. health assessment). Statistical comparisons of the laboratory reflectance measurements and selected optical vegetation indices reported here supports the hypothesis that the upper sunlit production crown can be sampled independent of the azimuth orientation. Consequently, results of sampling independent of the azimuth orientation should be representative for the entire sunlit portion of the crown, when interpreting remote sensing spectral images of very high spectral and spatial resolution.

The NDVI was used in this study to give supplementary information about the ratio of green and non-green biomass of examined shoots within the field of view of the VIRIS spectroradiometer. Almost identical values of NDVI for shoots of all branch orientations suggests that the samples are comparable in green biomass amount and suitable for evaluation of other optical indices. It is possible to imply that needle density on a shoot is the same for all needle age classes and branch orientations studied.

Identical patterns and similar values of the TCARI/OSAVI ratios for shoots of all branch orientations, closely related to the needle chlorophyll concentration ($R = -0.80$), fully confirm the statistical results obtained from the green foliar pigments analysis. Although a number of authors (Rock et al., 1988; Vogelmann et al., 1993) described a strong correlation between the wavelength position of the REIP and leaf chlorophyll concentrations, in present study the relationship between total chlorophyll needle concentrations and REIP was weaker ($R = 0.51$) then in the case of the TCARI/OSAVI ratio, but not significantly. Despite of some authors reporting changes in REIP position related also to leaf characteristics other than chlorophyll content (e.g. the so called "double-peak" feature of first derivative of vegetation reflectance; Zarco-Tejada et al., 2003), the REIP optical index can still be quite an efficient indicator of actual vegetation stress and physiological status. The fact that values of REIP shoots from all four azimuth orientations were similar indicate a similar physiological status of shoots from all branch orientations. Likewise the similar values of TM5/TM4 index and pattern of its values for all three needle age classes indicate comparable water status of shoots from branches of all orientations. Considering the results of the REIP and TCARI/OSAVI indices, total chlorophyll and soluble phenolic compounds analyses suggest similar environmental stress levels within the sunlit upper production crown part. Since none of the 20 trees samples were exhibiting any indications of elevated stress, the above measurements are reasonable.

The finding of identical reflectance properties for shoots from different azimuth-oriented branches in Norway spruce upper production crowns, together with homogenous chlorophyll, soluble phenolic concentrations and water content in needles, is significant for the quantitative remote sensing. At the leaf level this means that any needle sample of an even age class can be used to estimate leaf chlorophyll concentration by means of the spectrometric retrieval techniques, including the statistic-empirical method, as well as inversions of the physically based leaf radiative transfer models (e.g. the PROSPECT model; Jacquemoud and Baret, 1990; Jacquemoud et al., 1996). More implications can be found at the level of a vegetation canopy. For instance, most of the canopy analyses from very high spatial resolution data are performed from selected sunlit part of crown (due to a high level of spectral noise in the shadowed pixels; Malenovský et al., 2005). Canopy level radiative transfer (RT) models (e.g. Discrete Anisotropic Radiative Transfer; Gastellu-Etchegorry et al., 1996, 2004) are often parameterized by leaf or shoot measurements. This study's results propose that spruce needle parameters acquired from any azimuth orientation are representative for the whole forest stand canopy.

When dealing with shoot optical properties, the geometry of needle arrangement on the twig plays important role (Smolander and Stengerg, 2003). In the present study, we assumed similar "brush type" architecture of all the measured shoots, which enables the comparison of shoot spectral characteristics. On the other hand, the directional nature of reflectance should be further explored for a more general comparison and conclusions.

Based on our findings, any part of sunlit upper production tree crown can be used as a ground truth or calibration input into the remote sensing techniques based on reflectance designed for forest monitoring or estimation of the quantitative canopy parameters. However, even though all of the reflectance measurements and optical indices give consistent and convincing results, all of spectral characteristics of shoots were based on reflectance data. Full leaf optical properties, i.e. reflectance, transmittance and absorption, have to be investigated to test the full complement of spectral characteristics of a spruce upper crown part in horizontal direction. In contrast to the reflectance data, others (Rock et al., 1994; Roberts et al., 2004) found equal or more significant differences comparing transmittance of the leaf samples. Thus, the transmittance is quite an important parameter, describing the amount of photons passing through the leaf, and potentially taking part in the multiple scattering within a shoot and canopy. Considering this fact, that hemispherical transmittance is one of the input parameters to the RT models used for retrievals of the vegetation biochemical concentrations, there is a need for verification of the present results considering the full optical properties of needles.

In the present study we showed, based on a wide range of biochemical and structural needle properties frequently used for forest health status monitoring, that it is possible to apply random sampling within the upper production crown part of Norway spruce. We hope that our study will contribute to answering questions regarding sampling and questions regarding existing horizontal variability in these needle parameters. It is apparent that homogeneity in the needle parameters studied suggests that there are not microclimatic differences in the sun-exposed needles from upper production crown part of Norway spruce dependent on the azimuth orientation of the branch. This fact may indicate that the forest stands in the Northern hemisphere, which are not situated on significant slopes, receive comparable irradiance during the course of a day for different azimuth-oriented branches. After all, it seems that the vertical gradient of irradiance in a tree crown is the principal factor influencing biochemical and structural needle parameters.

5. Conclusion

The concentration of chlorophylls and carotenoids and amount of non-specific soluble phenolic compounds in even-aged needles from the upper production portion of a crown do not show any heterogeneity related to azimuth orientation of branches. Identical values of the selected optical vegetation indices (NDVI, TCARI/OSAVI, REIP and TM5/TM4) for shoots of all azimuth orientations suggest that the entire upper sunlit production part of the crown of Norway spruce canopy is homogenous relative to spectral reflectance, and so representative in support of remote sensing studies. Non-varying structural properties of even-aged needles from branches of the four azimuth orientations, supported by both conclusions mentioned above, raise the possibility of a fully random sampling design relative to the azimuth orientations of Norway spruce branches. This is also important for physiological studies when several comparable needle samples have to be obtained from one tree. All the findings support the general conclusion that information obtained from the sunlit upper production part of a tree crown is independent on branch azimuth orientation, and can be used as ground truth or calibration input in remote sensing techniques designed for forest monitoring or estimation of the quantitative canopy parameters.

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